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BENEFICIAL REGULATION OF TYPE I COLLAGEN AND MATRIXMETALLOPROTEINASE –1 EXPRESSION BY ESTROGEN, PROGESTERONE, AND ITS COMBINATION IN SKIN FIBROBLASTS.

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ABSTRACT

There is impaired wound healing and loss of type I collagen in skin aging, which can be improved by topical estrogen in vivo. The goal of this study was to determine the effects of estrogen, and progesterone and a combination of estrogen and progesterone as well, on the proliferation and the expression of type I collagen and matrixmetalloprotienase-1 (MMP-1, degrades collagen) in dermal fibroblasts (cells that synthesize collagen and MMP-1) in-vitro. Estrogen, progesterone, and its combination similarly and significantly inhibited cell proliferation and MMP-1 protein levels, and simultaneously stimulated type I collagen expression in the fibroblasts, indicating beneficial modulation.

INTRODUCTION

Skin aging is due to intrinsic (chronological) aging, photoaging (exposure to ultraviolet radiation), and hormonal aging (estrogen deficiency) (1). Skin aging manifests as atrophy of the extracellular matrix (ECM) and wrinkling, and delayed wound healing (2-4). The predominant structural ECM protein in the skin is type I collagen. With the aging of skin there is decrease in type I collagen and an increase in matrix metalloproteinase–1 (MMP-1), the enzyme that degrades type I collagen (5). The reduction in collagen expression with skin aging delays wound repair, but reduces scarring as well (3,4).

The delay in wound healing in the elderly can be significantly reduced by topical estrogen (3,4). Estrogen improves the mechanical properties of skin, and increases collagen deposition and transforming growth factor-beta (TGF-β) activity (3-4,6-9). TGF-β stimulates collagen and simultaneously inhibits MMP-1 in healing human fetal skin (10). The expression of the ECM proteins is primarily by skin fibroblasts.

In order to further understand the role of hormones in the prevention of extracellular matrix atrophy, we determined the effects of estrogen, progesterone, and a combination of estrogen and progesterone, respectively, on the proliferation, and the expression of type I collagen and MMP-1 in fibroblast cell cultures.

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MATERIALS AND METHODS

Dermal fibroblasts in replicates of four in 96 or 48 well plates were dosed with (i) estrogen (E) (estradiol 17-beta) at 0, 1, 10, 100, and 1000 nM, (ii) progesterone (P) (medroxyprogesterone) at 0, 0.1, 1, 10, and 100 uM, or (iii) a combination of estrogen (nM)/progesterone (uM) at 0, 1/0.1, 10/1, 100/10, and 1000/100 for 48 hours. These hormone concentrations (dissimilar doses) were based on reported physiological concentrations, with progesterone concentration being about 100 fold higher than estrogen. The cells were examined for cell proliferation. The media were examined for type I collagen, and MMP-1 proteins.

Dermal fibroblasts in replicates of four in 96 or 48 well plates were also dosed with identical doses (1, 10, 100, 1000, and 10000 nM) of estrogen, progesterone, or a combination of the hormones, and examined for cell proliferation, and expression of type I collagen and MMP-1. The results obtained were similar to the dissimilar (physiological) doses and hence data corresponding to the physiological doses are being represented in this paper.

Cell Proliferation

The reduction of a MTS mix (Promega) to give a colored product, in proportion to cell number, was measured spectrophotometrically at 490nm.

Protein levels

The secretion of type I collagen or MMP–1 was measured using indirect ELISA (ELISAmate, Kirkegaard and Perry). 100µl of medium from each sample was added to an independent well of a 96 well plate for 24 hr at 4°C. The wells were blocked with bovine serum albumin, and then incubated with type I collagen or MMP–1 antibody for 1 hr at room temperature. The plate was washed thoroughly with wash buffer, incubated with secondary antibody linked to peroxidase for 1 hr at room temperature, washed with wash buffer thoroughly, and subsequently incubated with peroxidase substrate until color development which was measured spectrophotometrically at 405nm.

Data Analysis and Representation

The data were statistically analyzed for significance ($p<0.05$) by ANOVA and student t-tests at the 95% confidence interval. Data are represented as a percent of control (represented at 100%), with control being cells not exposed to the hormones.

RESULTS

Inhibition of Cell Proliferation by Hormones

Figure 1 demonstrates the significant inhibition of cell proliferation by estrogen and progesterone, independently and in combination, relative to control cells ($p<0.05$). The inhibitory effect of estrogen, progesterone, and the combination of estrogen and progesterone were not significantly different from each other ($p>0.05$).

Relative to control cell number (represented as 100%), cell numbers in fibroblasts dosed with 1, 10, 100, and 1000 nM of estrogen were 80%, 84%($p<0.05$), 71%($p<0.05$) and 67%($p<0.05$) respectively, with 0.1, 1, 10, and 100 uM progesterone were 71%($p<0.05$), 81%($p<0.05$), 80%($p<0.05$) and 73%($p<0.05$) respectively, and with a combination of estrogen (nM)/progesterone (uM) at 1/0.1, 10/1, and 100/10 were 70%($p<0.05$), 70%($p<0.05$) and 74%($p<0.05$) respectively.

Inhibition of MMP-1 Expression by Hormones

Figure 2 shows the inhibition of MMP-1 expression by estrogen, progesterone, and the combination of estrogen and progesterone relative to control cells ($p<0.05$), but not relative to each other ($p>0.05$).

Relative to MMP–1 protein level in the medium of control cells (100%), the MMP-1 protein levels in the media of fibroblasts dosed with 1, 10, 100, and 1000 nM of estrogen were 98%, 65%($p<0.05$), 62%($p<0.05$) and 55%($p<0.05$) respectively, with 0.1, 1, 10, and 100 uM progesterone were 76%, 73%($p<0.05$), 66%($p<0.05$) and 70%($p<0.05$) respectively, and with a combination of estrogen (nM)/progesterone (uM) at 1/0.1, 10/1, 100/10 and 1000/100 were 53%($p<0.05$), 43%($p<0.05$), 43%($p<0.05$) and 47%($p<0.05$) respectively.

Stimulation of Type I Collagen Expression by Hormones

Figure 3 demonstrates the stimulation of type I collagen by the hormones, relative to control cells ($p<0.05$). The effects of estrogen, progesterone and the combination of estrogen and progesterone were statistically similar to each other ($p>0.05$).

Relative to type I collagen protein level in the medium of control cell (100%), the protein levels of type I collagen in the media of fibroblasts dosed with 1, 10, 100, and 1000 nM of estrogen were 169%, 162%($p<0.05$), 142%($p<0.05$) and 235%($p<0.05$) respectively, with 0.1, 1, 10, and 100 uM progesterone were 244%($p<0.05$), 270%($p<0.05$), 232%($p<0.05$) and 230%($p<0.05$) respectively, and with a combination of estrogen (nM)/progesterone (uM) at 1/0.1, 10/1, 100/10, and 1000/100 were 321%($p<0.05$), 345%($p<0.05$), 343%($p<0.05$) and 320%($p<0.05$) respectively.

DISCUSSION

Skin is an important target organ for estrogen and progesterone, and the receptors for these hormones have been localized to skin fibroblasts and keratinocytes (11).

Topical application of estrogen (5-50mM to ovariectomized animals, and 25uM estrogen to human skin) accelerates the wound healing process in aged skin, similar to young skin, with regards to reepithelilization, TGF-β activity, and collagen deposition. The mechanisms by which the hormones improve skin quality and wound healing have been implicated to increased TGF-β activity, scavenging of superoxides, and an altered inflammatory response (12-15).

Our in-vitro studies support, and extend, the reported observations on the positive effects of estrogen on the skin. It also demonstrates progesterone, and a combination of estrogen and progesterone to have effects similar to that of estrogen. We observed inhibition of MMP-1 and the stimulation of type I collagen from almost the smallest doses of estrogen (10nM) or progesterone (0.1uM) used to the highest doses (1000nM of estrogen and/or 100uM of progesterone) tested. Estrogen and progesterone, independently and in combination, had similar effects implicating synergy, and similar mechanisms of action.

The hormones inhibited cell proliferation, at 10nM to 1000nM of estrogen or/and 0.1 uM to 100uM of progesterone. Estrogen and progesterone, from 1pM to 1mM, have been reported to inhibit fibroblast cell proliferation, independently of the stimulation of TGF-β activity (3).

The hormones, at 10nM to 1000nM of estrogen or/and 0.1 uM to 100uM of progesterone, inhibited MMP-1 protein levels in the dermal fibroblasts. MMP-1 is the enzyme that degrades type I collagen, and hence the inhibition of MMP-1 may further accentuate the collagen deposition by the hormones. This is the first report of the inhibition of MMP-1 by estrogen, and progesterone in skin.

The basal levels of MMP-1 are reported to be higher in aged skin, relative to young skin (16). Estrogen and progesterone may, additionally to increasing collagen, improve the mechanical properties of the skin by inhibiting MMP-1 expression. Physiological doses of estrogen (1nM) and progesterone (100nM) have been reported to inhibit MMP-1 in endometrial fibroblasts, and progesterone to act in concert with TGF-β in its inhibition of MMP-1 (17,18).

Estrogen, progesterone, and the combination of estrogen and progesterone (at 10nM to 1000nM of estrogen or/and 0.1 uM to 100uM of progesterone) stimulated type I collagen protein levels in the dermal fibroblast cultures. Estrogen has been reported to stimulate collagen in vivo (3,4). Our studies indicate estrogen, and progesterone as well, can directly stimulate type I collagen expression in the dermal fibroblasts.

Overall, these studies indicate that estrogen, progesterone act synergistically on skin fibroblasts to simultaneously stimulate type I collagen and inhibit MMP-1. It supports topical application of estrogen and progesterone as beneficial to wound healing, and skin health.

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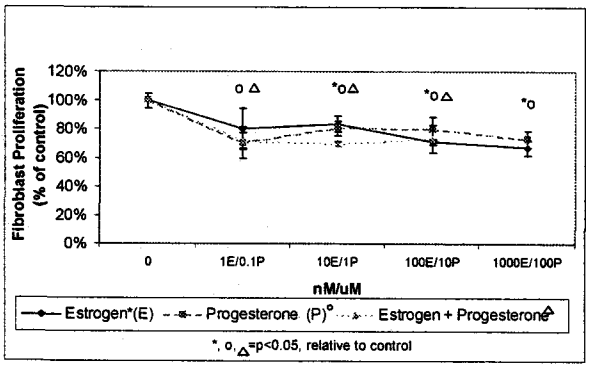

Figure 1: Inhibition of Cell Proliferation by Estrogen, Progesterone, and a Combination of Estrogen/Progesterone.

Fibroblasts were exposed to estrogen (1-1000 nM; blue straight line), progesterone (0.1-100 uM; purple dashed line), and a combination of estrogen (nM)/progesterone (uM) (1/0.1-1000/100; green dotted line) for 48 hours and analyzed for cell proliferation. Effects of hormones are represented as % of control (no hormone, 0 nM/uM) at 100%. Significant differences ($p<0.05$) from control are represented as * for estrogen, o for progesterone and △ for estrogen + progesterone. Error bars represent standard deviation, n=4.

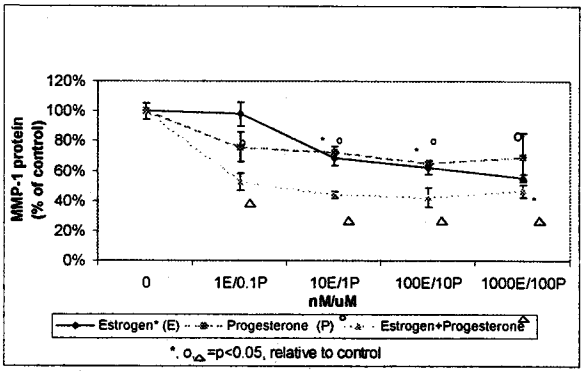

Figure 2: Inhibition of Matrixmetalloproteinase-1 (MMP-1) Expression by Estrogen, Progesterone, and a Combination of Estrogen/Progesterone.

MMP-1 protein levels in the media of fibroblasts exposed to estrogen (1-1000 nM; blue straight line), progesterone (0.1-100 uM; purple dashed line), and a combination of estrogen (nM)/progesterone (uM) (1/0.1-1000/100; green dotted line) for 48 hours are represented as % of control (no hormone, 0 nM/uM) at 100%. Significant differences ($p<0.05$) from control are represented as * for estrogen, o for progesterone and △ for estrogen + progesterone. Error bars represent standard deviation, n=4.

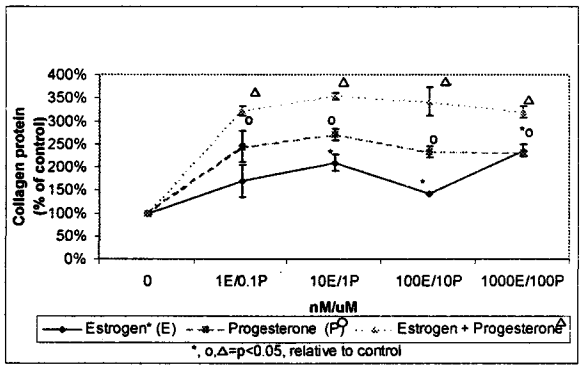

Figure 3: Stimulation of Expression of Type I Collagen by Estrogen, Progesterone, and a Combination of Estrogen/ Progesterone.

Type I collagen protein levels in the media of fibroblasts exposed to estrogen (1-1000 nM; blue straight line), progesterone (0.1-100 uM; purple dashed line), and a combination of estrogen (nM)/progesterone (uM) (1/0.1-1000/100; green dotted line) for 48 hours are represented as % of control (no hormone, 0 nM/uM) at 100%. Significant differences ($p<0.05$) from control are represented as * for estrogen, o for progesterone and △ for estrogen + progesterone. Error bars represent standard deviation, n=4.

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